Active Site Polymerase Inhibitor Nucleotides (ASPINs)

Robert G. Gish, MD
Disclosures

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Challenges of Treating Chronic HBV

- Chronic HBV is currently an incurable disease that requires lifelong therapy to maintain viral suppression in most patients\(^1\)

- A “functional cure” for chronic HBV infection results in the loss of HBsAg and HBV DNA off treatment, while a “complete cure” will require elimination of cccDNA, the stable archive of HBV DNA that persists in infected hepatocytes\(^2,3\)

- Current nucleos(t)ide analogues effectively control viral replication but do not eliminate cccDNA and rarely achieve HBsAg seroclearance\(^1,2\)

- Antiviral agents with novel MOAs that can complement the antiviral activity of nucleos(t)ide analogues are needed to achieve a curative regimen for chronic HBV infection

cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MOA, mechanism of action.

MOA of Traditional Chain-Terminating Nucleos(t)ide Analogues

- Anti-HBV nucleos(t)ide analogues compete with endogenous deoxynucleoside triphosphates for incorporation into the growing HBV DNA chain to inhibit DNA synthesis by HBV polymerase.\(^1\)
  - Entecavir inhibits protein priming through competition with deoxyguanosine triphosphate, the native nucleoside that initiates protein priming.\(^2\)
  - TDF lacks a 3'-OH group, which prevents binding of additional nucleotides.\(^1\)

DR1, direct repeat 1; HBV, hepatitis B virus; MOA, mechanism of action; NRTI, nucleos(t)ide reverse transcriptase inhibitor; OH, hydroxyl; pgRNA, pregenomic RNA; RNase H, ribonuclease H; RT, reverse transcription domain; TDF, tenofovir disoproxil fumarate; TP, terminal protein domain.

MOA of ASPINs

- ASPINs are polymerase inhibitors that are differentiated from traditional chain-terminating nucleos(t)ide analogues by their unique MOA\(^1\)
- ASPINs noncompetitively distort the HBV polymerase active site to completely inhibit all HBV polymerase functions\(^1,-3\)
  - ASPINs inhibit **protein priming** independently of the initiating nucleotide and without being used as a substrate\(^3\)
  - ASPINs inhibit **primer elongation** and **DNA synthesis** without being incorporated into the DNA chain\(^3\)

ASPIN, active site polymerase inhibitor nucleotide; DR1, direct repeat 1; HBV, hepatitis B virus; MOA, mechanism of action; NRTI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; RNase H, ribonuclease H; RT, reverse transcription domain; TDF, tenofovir disoproxil fumarate; TP, terminal protein domain.

Clevudine Demonstrated Prolonged Anti-HBV Activity in the Woodchuck HBV Model

• In a woodchuck model of chronic HBV infection, clevudine demonstrated potent anti-HBV activity and prolonged off-treatment viral load suppression\textsuperscript{1,2}

• WHV DNA, replication intermediates, and WHsAg levels were reduced after 4 weeks of treatment with clevudine 10 mg/kg, with continued decline of WHsAg for 4 weeks off treatment\textsuperscript{2}

The Prolonged Anti-HBV Activity in the Woodchuck Was Associated with cccDNA Reductions

- Clevudine treatment of woodchucks resulted in substantial reductions in intrahepatic cccDNA\(^1,2\)
- After 30 weeks of clevudine treatment, intrahepatic cccDNA declined to between 1.2% and 5.4% of pretreatment levels\(^2\)
  - The average cccDNA copy number decreased from 19 to 63 copies/cell before treatment to <1 copy/cell after 30 weeks of treatment
  - Reductions in cccDNA corresponded with hepatocyte cell death, supporting a mechanism of hepatocyte turnover

ccaDNA, covalently closed circular DNA; HBV, hepatitis B virus; \(t_{1/2}\), half-life.


Reductions in cccDNA With 30 Weeks of Clevudine 10 mg/kg Treatment\(^2\)

<table>
<thead>
<tr>
<th>Woodchuck</th>
<th>Percent cccDNA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

**Week**
- **Woodchuck 1**
  - \(t_{1/2} = 50\) days

- **Woodchuck 2**
  - \(t_{1/2} = 33\) days

- **Woodchuck 3**
  - \(t_{1/2} = 50\) days

- **Woodchuck 4**
  - \(t_{1/2} = 33\) days

\(^a\)Woodchuck 2 died after 4 weeks of treatment.
Clevudine Demonstrated Potent and Prolonged Anti-HBV Activity in Clinical Studies

- Clevudine is a first-generation ASPIN that demonstrated potent and prolonged HBV suppression in phase 2 clinical studies\(^1-^3\)
- Clevudine treatment for 28 or 90 days in CHB patients led to prolonged reduction in HBV DNA from baseline post-treatment


ASPIN, active site polymerase inhibitor nucleotide; CHB, chronic hepatitis B; HBV, hepatitis B virus.
Clevudine Demonstrated Potent and Prolonged Anti-HBV Activity in Clinical Studies

- Clevudine demonstrated potent and prolonged HBV suppression in phase 3 clinical studies\(^1,2\)
- Clevudine demonstrated similar virologic response rates to entecavir after 1 year of treatment\(^3\)
- Clinical development of clevudine was halted after long-term treatment was associated with reversible skeletal myopathy in a small number of patients (1.7% to 3.9%)\(^4-6\)

### Summary of Phase 3 Clinical Trials Evaluating Once-Daily Clevudine Monotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>HBeAg status</th>
<th>Treatment group</th>
<th>n</th>
<th>Treatment duration</th>
<th>HBV DNA reduction, (\log_{10}) copies/mL</th>
<th>Undetectable HBV DNA, %</th>
<th>HBV DNA reduction, (\log_{10}) copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoo 2007a(^2)</td>
<td>HBeAg+</td>
<td>Clevudine 30 mg</td>
<td>182</td>
<td>24 weeks</td>
<td>−5.10</td>
<td>59</td>
<td>−2.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>61</td>
<td></td>
<td>−0.27</td>
<td>0</td>
<td>−0.62</td>
</tr>
<tr>
<td>Yoo 2007b(^3)</td>
<td>HBeAg−</td>
<td>Clevudine 30 mg</td>
<td>63</td>
<td>24 weeks</td>
<td>−4.25</td>
<td>92</td>
<td>−3.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>23</td>
<td></td>
<td>−0.48</td>
<td>0</td>
<td>−0.66</td>
</tr>
</tbody>
</table>

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

ATI-2173 is a Next Generation ASPIN

- ATI-2173 contains a phosphoramide on the 5'-hydroxyl of clevudine that undergoes enzymatic and nonenzymatic reactions to yield the same 5'-triphosphate as clevudine.
ATI-2173 Demonstrated Prolonged Off-Treatment Viral Load Responses

- Sustained viral load responses through 24 weeks off treatment were observed with ATI-2173 treatment for 28 days
  - After 24 weeks of clevudine treatment, reductions of 2.0 to 3.1 log_{10} IU/mL in HBV DNA were observed\(^1,2\)
- In patients receiving ATI-2173, HBV DNA gradually increased toward baseline levels without ALT flares during the 24-week off-treatment follow-up period
  - 1 patient in the 25-mg group remained BLQ for 24 weeks off treatment

ALT, alanine aminotransferase; BLQ, below the limit of quantification; HBV, hepatitis B virus; SD, standard deviation.
Prolonged HBV RNA Suppression With ATI-2173 Demonstrates Potential cccDNA Activity

- HBV RNA decreased on treatment in all patients with ATI-2173 25 or 50 mg, with continued decreases for 4 weeks off-treatment, similar to HBV DNA
- Sustained off-treatment reductions in HBV RNA were observed with ATI-2173
  - All patients receiving ATI-2173 25 or 50 mg were BLQ by week 4 (W4) off treatment and slowly increased toward baseline levels by week (W24) off treatment
- No pattern of change in HBV RNA was observed in the placebo group

### Mean change from baseline over time

<table>
<thead>
<tr>
<th></th>
<th>Placebo, N=6</th>
<th>ATI-2173 25/50 mg, N=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>With BLQ, n (% of N)</td>
<td>1 (17)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>With BLQ, n (% of N)</td>
<td>0 (0)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>ATI-2173 25/50 mg, N</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>With BLQ, n (% of N)</td>
<td>5 (63)</td>
<td>3 (43)</td>
</tr>
</tbody>
</table>

**Mean change from baseline over time**

<table>
<thead>
<tr>
<th>Day</th>
<th>W4 (D55)</th>
<th>W12 (D111)</th>
<th>W24 (D195)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo, N=6</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ATI-2173 25/50 mg, N=8</td>
<td>1 (0)</td>
<td>7 (100)</td>
<td>3 (43)</td>
</tr>
</tbody>
</table>

BLQ, below the limit of quantification; cccDNA, covalently closed circular DNA; D, day; HBV, hepatitis B virus; W, week.

*BLQ = HBV RNA <10 copies/mL. Patients with HBV RNA that was not detectable at baseline and throughout most of the study were excluded from the HBV RNA analysis (n=1 in placebo group; n=2 in ATI-2173 group).*

One patient in the ATI-2173 group had missing data.
Future of HBV Cure Regimens

- Because of its unique MOA and prolonged off-treatment responses, ASPINs can be used as the cornerstone of a combination regimen for HBV cure.
- ASPINs demonstrate additive-to-synergistic activity in vitro with nucleos(t)ide analogues or capsid inhibitors\(^1\),\(^2\).
- Combining an ASPIN with a chain-terminating nucleos(t)ide analogue could shut down the HBV polymerase more potently.
- The next-generation ASPIN ATI-2173 is currently being evaluated in a phase 2a study in combination with TDF or an siRNA.

**ASPIN, active site polymerase inhibitor nucleotide; ETV, entecavir; HBV, hepatitis B virus; MOA, mechanism of action; siRNA, small interfering RNA; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.**

Conclusions

- ASPINs inhibit all HBV polymerase functions without being incorporated into HBV DNA, demonstrating a distinct MOA from traditional chain-terminating nucleos(t)ide analogues.
- ASPINs demonstrate potent anti-HBV activity and prolonged off-treatment viral suppression.
  - Viral rebound following discontinuation of ASPIN treatment is slow, reducing the potential for hepatic flare.
- ASPINs decrease cccDNA copy number in the woodchuck HBV model and reduce cccDNA biomarkers in man.
- Combining ATI-2173 with a chain-terminating nucleos(t)ide analogue could shut down the HBV polymerase more potently, potentially leading to a higher functional cure rate.
- Future combination regimens for HBV cure should include ASPINs because of their unique MOA and prolonged off-treatment responses.

ASPIN, active site polymerase inhibitor nucleotide; HBV, hepatitis B virus; MOA, mechanism of action.
Acknowledgments

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